The synthesis of GdB-texaphyrins

as novel multipurpose cancer drugs

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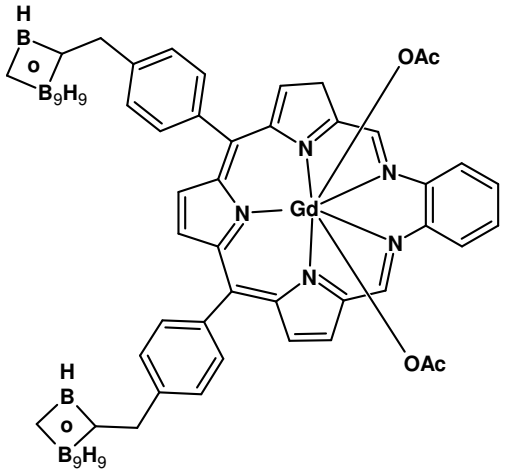
Abstract

Texaphyrins are a synthetic class of porphyrin analogues that contain a metal atom complexed at their centers. This class of compounds selectively accumulates in cancer cells, thus creating a promising opportunity for medicinal chemists to synthesize novel anti-cancer agents. Texaphyrins may become especially valuable as anti-cancer agents by synthetically including atoms such as gadolinium and boron into their structures. The presence of either of these elements allows the compounds to be used in Neutron Capture Therapy (NCT), a non-invasive experimental treatment procedure for cancer.



While texaphyrins are promising novel agents for use in oncology, better synthetic methods for developing and optimizing these compounds are still needed. The purpose of this study is to find effective synthetic methods for the development of a texaphyrin that could be used in cancer therapy and diagnosis. Two such gadolinium- and boron-containing texaphyrins (GdB-texaphyrins) have already been successfully synthesized in our laboratories and are currently undergoing pre-clinical trials. This project has accomplished the synthesis of the direct pre-cursor to a specific GdB-texaphyrin (pictured on this page), and the compound's complete synthesis will continue to be pursued in our laboratories. This study has therefore provided valuable data about the pursued synthetic pathway to help researchers continue to advance the synthesis of this novel class of anti-cancer agents.

Introduction

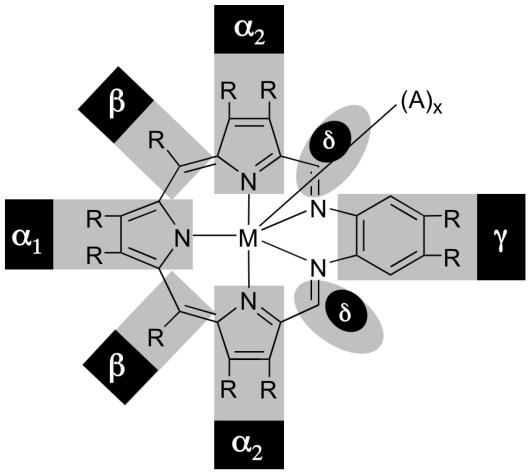
Texaphyrins are large, macrocyclic compounds that contain a lanthanide metal atom complexed at their centres. This class of compounds selectively accumulates in cancer cells[1], and our synthesis of texaphyrins attempts to equip them with the necessary means to be used in cancer diagnosis and treatment.

This is accomplished by the inclusion of gadolinium and boron into the texaphyrin. The presence of boron and/or gadolinium allows the compound to be used as the chemical agent in Neutron Capture Therapy (NCT), a non-invasive, chemo-radiotherapeutic modality for the treatment brain tumors[2]. NCT involves exposing boron- and/or gadolinium-infused tumour cells to neutrons. Both elements and the neutrons are alone harmless to biological tissue, but form lethal particles and radiation that easily kill cells when they come in contact with each other. It is important to NCT that both boron and/or gadolinium accumulate only in tumour cells so that the tumour will be destroyed while nearby healthy tissue is left unharmed. This can be accomplished by linking these elements to texaphyrins, since they selectively accumulate in tumour tissue but not in healthy tissue.

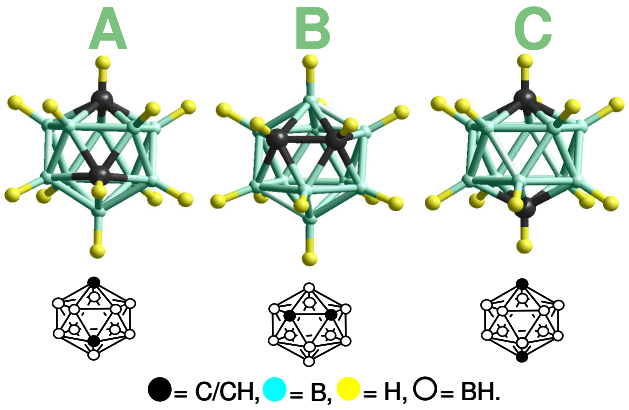
The presence of gadolinium in these compounds also allows their distribution in living tissue to be determined by Magnetic Resonance Imaging (MRI), thus making these compounds also useful to the *diagnosis* of cancer. Texaphyrins may also have potential uses in other cancer treatment and diagnostic procedures, such as Near Infrared Imaging[1].

Structurally, texaphyrins are expanded porphyrin-like compounds that contain a metal atom at their center (in this case gadolinium) surrounded by one benzene unit and

three pyrrole units. There are several possibilities to incorporate boron into these compounds. In principle, texaphyrins contain four possible subunits (α, β, γ, δ) that can contain boron (see figure). In this project, we chose to bind a carborane cluster, containing ten boron atoms, to an aromatic aldehyde (used as the "β" subunit), which is then used to connect the benzene and pyrrole units of the texaphyrin. Previously synthesized GdB-texaphyrins that have included the boron-rich carborane cages at the α_2 subunit are now being tested in our collaborator's lab for their chemical and biological properties.

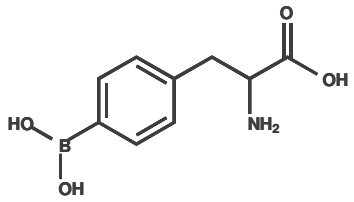


The aforementioned carborane clusters have been the preferred boron moieties in BNCT compound development because of their chemically modifiable nature, structural and physicochemical versatility, high boron content, and stability[3]. Each cluster contains ten boron atoms and two carbon atoms arranged in an *ortho*, *meta*, or *para* conformation (see figure below). After preliminary attempts at the synthesis using *ortho*-carborane and previous experience using *para*-carborane, it was decided that *meta*-carborane would be used in this project. *Meta*-carborane appeared to afford the highest-yielding reactions in this synthesis, presumably due to it being more stable than the *ortho* isomer and also having a somewhat lower potential to



create dimers than the *para* isomer.

The two boron-containing compounds that are currently being used as chemical agents in BNCT are boronphenylalanine (BPA) and sodium borocaptate (BSH)[2] (structures at right). BNCT has been successful in shrinking tumors using these agents, but better agents need to be synthesized[2,5]. Challenges in synthesizing better BNCT agents include achieving improved tumor selectivity and sufficient boron concentrations within tumors, estimated to be ~20 µg/g tumor[2], while avoiding systemic toxicity. GdB-Texaphyrins have the potential to be better BNCT agents because they contain more boron per molecule, show excellent tumor specificity, and exhibit low system toxicity[1].



Boronophenylalanine (BPA)

Sodium Borocaptate (BSH)

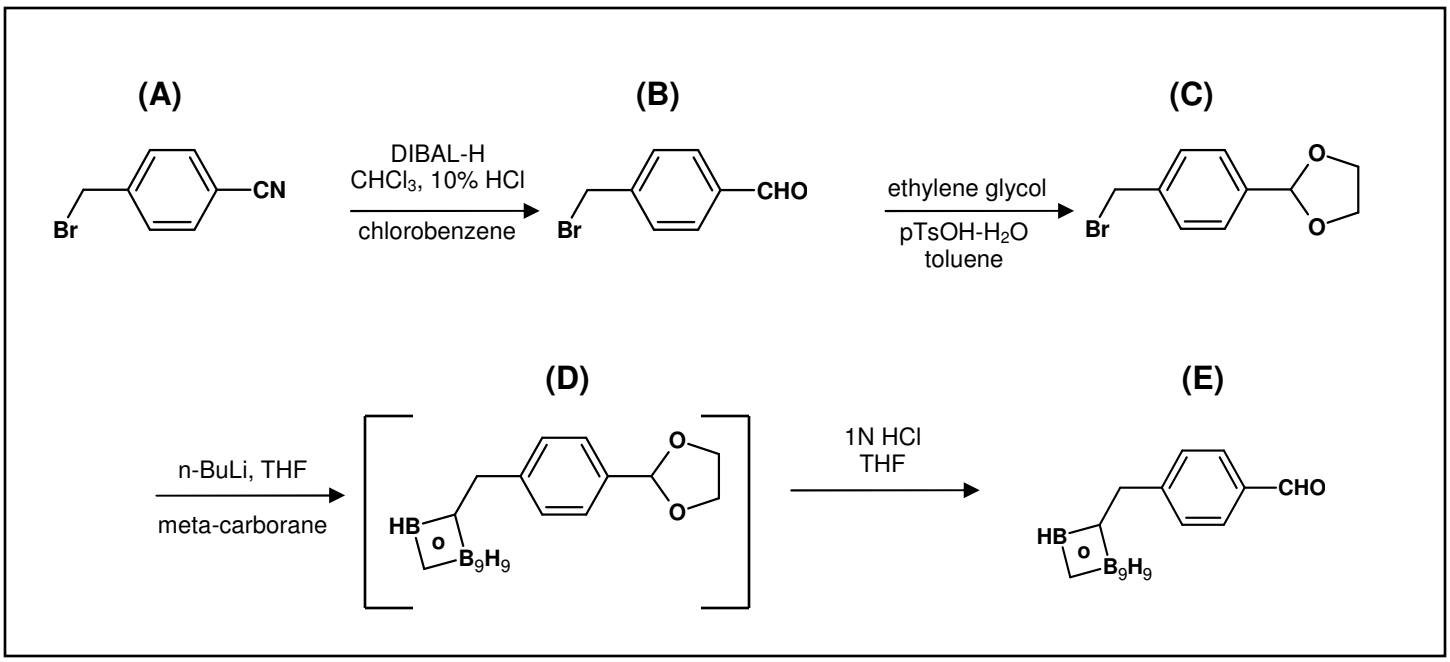
Specific Aim

The specific aim of this project is to further develop synthetic strategies to generating GdB-texaphyrins that can be used in cancer therapy and diagnostics.

Synthetic Strategies

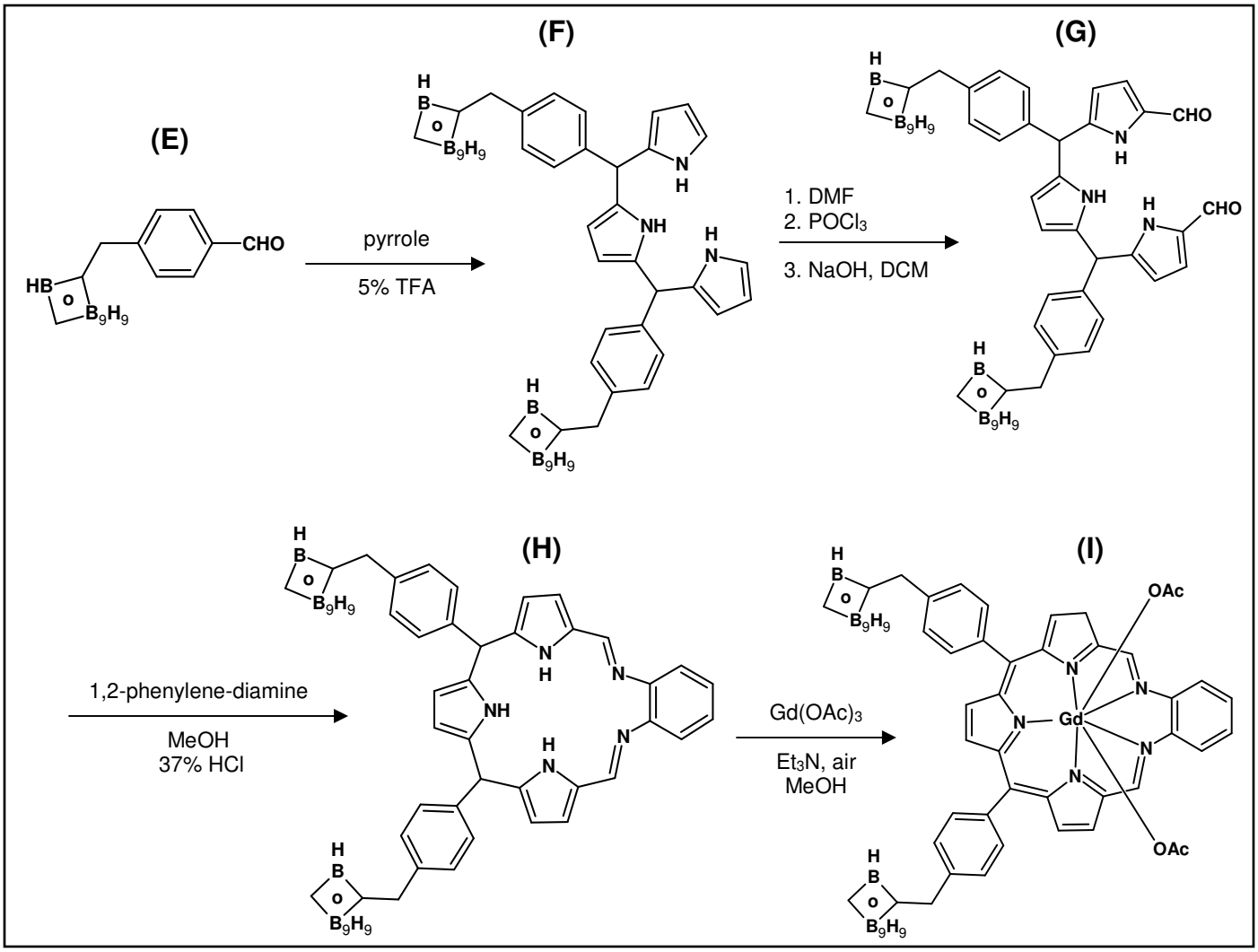
The starting material, *p*-(bromomethyl)benzonitrile, is commercially available (compound A). p-(Bromomethyl)benzonitrile (A) is oxidized to an aldehyde (B) using DIBAL-H (diisobutylaluminum hydride). The aldehyde function is then protected as an acetal (C) so that the boron moiety can be incorporated. *meta*-Carborane is introduced in the next step by substituting bromine (D). The acetal protective group is then removed, forming the carboranyl "aldehyde precursor" (E).

Scheme 1: Precursor Synthesis:



Then, a “pyrrole trimer” (F) is generated. It consists of two “aldehyde precursors” (E) and three pyrrole units. This was specified at the beginning of the project as the target molecule (F). A Vilsmeier-Haack reaction is then performed to formylate the two available pyrrole units (G). The formylating agent is formed *in situ* from DMF and oxalyl chloride, and forms an α-chloro amine with the available pyrrole units via electrophilic aromatic substitution. The amine is then rapidly hydrolyzed during workup to give the aldehyde (G). A Schiff base reaction is then performed to “cyclize” the molecule (this is a relatively unstable intermediate) (H). Finally, the gadolinium is complexed at the molecule’s center, creating the GdB-texaphyrin (I).

Scheme 2: Cyclization Synthesis

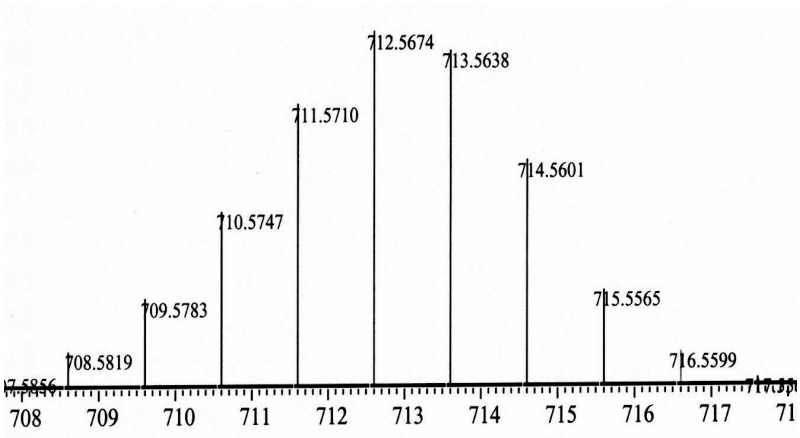


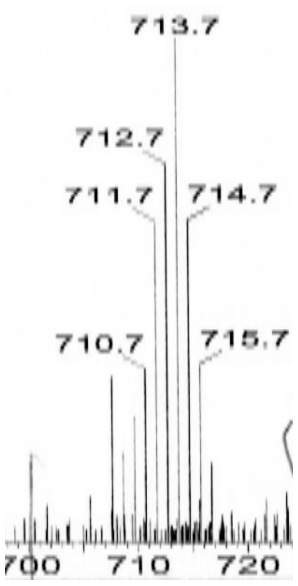
Results and Discussion

Compound B was generated in ~90% yields after purification via column chromatography with hexane and ethyl acetate in a 9:1 ratio. Crude compound C was obtained in high yields, and was used in the next step without further purification to avoid premature de-protection of the aldehyde.

Compound D was obtained in approximately 45% yield, and was de-protected to compound E without further purification due to the propensity of the acetal group to de-protect during purification. This resulted in a total yield of approximately 34% for the C-E synthetic steps. Several aliquots of Compound E from multiple reaction runs were collectively purified via column chromatography and then characterized via ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

Compound F was believed to be generated in 25% yield as purple crystals, but with an impurity that made it difficult to retrieve conclusive Nuclear Magnetic Resonance (NMR) spectra. The theoretical (left) and actual (right) mass spectra of compound F are below. They are very similar and so we proceeded with the synthesis under the



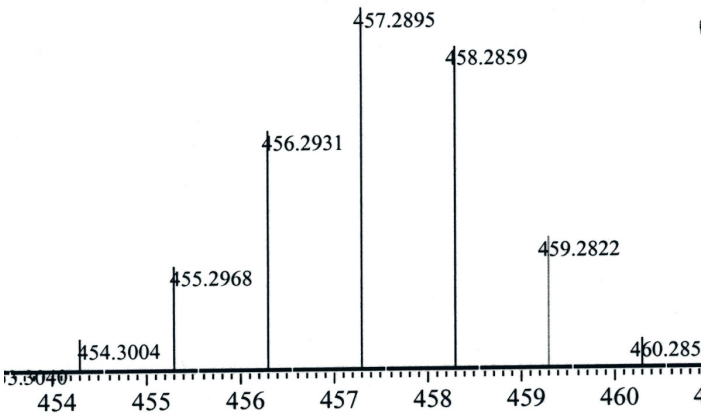


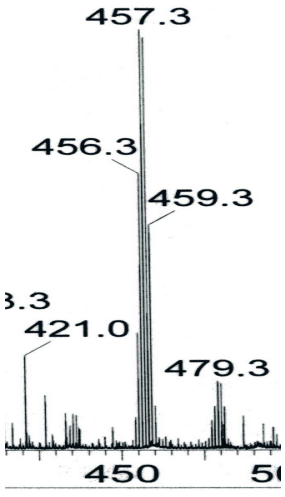
The theoretical spectrum (above left) shows the dominant peak at about molecular weight 712.6, and our sample (right) generated its dominant peak at molecular weight 713.7.

The theoretical spectrum was generated using "Isotope Pattern Calculator v4.0," developed by Junhua Yan.

assumption that the “pyrrole trimer” had been synthesized. When the synthesis of compound G was attempted, however, dimeric pyrrole di-aldehyde was found to be predominantly formed instead of the target trimeric pyrrole di-aldehyde, and in 13% yield. The NMR data for both compound F and compound G was difficult to interpret due to impurities, however, mass spectroscopy data was more helpful in characterizing these compounds.

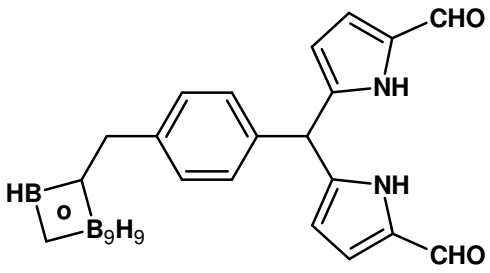
The structure and corresponding mass spectra of the proposed “dimeric pyrrole di-aldehyde” are shown below. It is postulated that the impurity noted in the trimerization reaction (the formation of compound F) was a pyrrole dimer, instead of the target trimer (F). It is also believed that the reaction conditions of the following step (the formation of compound G) favored the formylation of the dimer over that of the trimer, resulting in the dimeric pyrrole di-aldehyde (shown below) being the dominant product formed instead of the target trimeric pyrrole di-aldehyde (compound G).





The theoretical spectrum (above left) shows the dominant peak at about molecular weight 457.3, and our sample (right) generated its dominant peak at molecular weight 457.3.

The theoretical spectrum was generated using “Isotope Pattern Calculator v4.0,” developed by Junhua Yan.



Future attempts to complete this synthesis should focus on diligently purifying the "pyrrole trimer" (compound F), and on adjusting reactant ratios in the following reaction to favor the formation of the *trimeric* pyrrole di-aldehyde (compound G), instead of the *dimeric* pyrrole di-aldehyde that predominated.

Conclusion and Outlook

Two novel GdB-texaphyrins have already been synthesized in our laboratories and are currently undergoing pre-clinical trials. This project has accomplished the synthesis of pre-cursors to a specific GdB-texaphyrin (compound I), and its complete synthesis will continue to be pursued in our laboratories. This will be followed by its biological and pre-clinical evaluation.

This study has therefore provided valuable data about the pursued synthetic pathway to help researchers continue to advance the synthesis of this novel class of anti-cancer agents.

Experimental Procedures

General experimental procedures. ^{1}H-and ^{13}C-NMR spectra were obtained on a Bruker 400 MHz FT-NMR instrument. Chemical shifts are reported in parts per million (ppm). The coupling constants are reported in Hertz (Hz). Compound visualization on Silica Gel 60 F254 pre-coated TLC plates (0.25 mm layer thickness) (Merck, Darmstadt, Germany) was attained by UV light. Reagent grade solvents were used for column chromatography using Silica gel 60, particle size 0.040-0.063 mm (Merck, New Jersey). Anhydrous chlorobenzene and pyrrole and other reagent grade chemicals were

obtained from commercial vendors and used as such. Low- and High-resolution electrospray ionization (LR- and HR-ESI) mass spectra were recorded on a Micromass Q-Tof II electrospray mass spectrometer and a Micromass LCT electrospray mass spectrometer at The Ohio State University Campus Chemical Instrumentation Center (OSU-CCIC). The theoretical spectra were generated using "Isotope Pattern Calculator v4.0," developed by Junhua Yan.

***p*-(Bromomethyl)-benzaldehyde (Compound B).** To a stirred solution of *p*-(bromomethyl)benzonitrile (3.00 g, 15.3 mmol) in anhydrous chlorobenzene (50 mL) was added a 1M solution of DIBAL-H in hexanes (24.5 mL, 24.5 mmol) over a 20 minute period at 0°C. The reaction mixture was stirred for 2.5 hours at 0°C, diluted with dichloromethane, and treated with a 10% aqueous HCl solution. The reaction mixture was then extracted with dichloromethane, and washed with brine. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified via column chromatography with hexane and ethyl acetate in a 9:1 ratio to give the pure product in 93% yield (2.83 g). ^{1}H NMR ($CDCl_3$): δ 10.05 (s, 1H, -CHO), δ 7.90 (d, 2H, -Ph), δ 7.59 (d, 2H, -Ph), δ 4.55 (s, 2H, Br-$\underline{CH_2}$-Ph)

2-[4-(Bromomethyl)phenyl]-1,3-dioxolane (Compound C). A mixture of *p*-(bromomethyl)-benzaldehyde (compound B) (1.00 g, 5.2 mmol), ethylene glycol (1.1 mL, 1.25 g, 20.1 mmol), and a catalytic amount of *p*-toluenesulfonic acid in toluene (50 mL) was refluxed in a Dean Stark apparatus for 6 hours. The reaction mixture was then cooled to room temperature, extracted with cold water and EtOAc, washed with brine,

and dried over Na_2SO_4. Subsequently, the organic phase was evaporated. The resulting crude mixture (1.75 g) was then used in the next step with out purification to avoid premature de-protection of the aldehyde. ^1H NMR ($CDCl_3$): δ 7.44 (d, 2H, -Ph), δ 7.39 (d, 2H, -Ph), δ 5.79 (s, 1H, Ph-C<u>H</u>-acetal), δ 4.47 (s, 2H, Br-<u>CH_2</u>-Ph), δ 4.05 (m, 4H, acetal)

2-[4-(Carboranylmethyl)phenyl]-1,3-dioxolane (Compound D). To a solution of *m*-carborane (2.60 g, 18.0 mmol) in THF (60 mL) was added drop-wise a 2.5 M solution of BuLi in hexanes (6.6 mL, 16.5 mmol) at 0℃ under anhydrous Ar. The reaction mixture was stirred at rt for 1.5 hours. The 2-[4-(bromomethyl)phenyl]-1,3-dioxolane synthesized in the previous step (compound C) (3.65 g, 15.0 mmol) was diluted slightly with THF before being added to the reaction mixture over a 2 minute period. The reaction mixture was then stirred overnight, extracted with ether, and washed with brine. The organic phase was dried over Na_2SO_4, filtered, and evaporated. The resulting residue (compound D in approximately 45% yield, 2.07 g) was used in the next reaction without further purification (see Results and Discussion).

***p*-(Carboranylmethyl)-benzaldehyde (Compound E).** Compound D (2.07 g, 6.76 mmol) was stirred overnight with 1N HCl (6 mL) in THF (60 mL). The reaction mixture was then extracted with ether, washed with brine, and dried over Na_2SO_4. Subsequently, the organic phase was evaporated to generate compound E in 91% crude yield (1.55 g). Combined portions of this crude product from multiple reaction attempts were combined at a later date and purified via column chromatography with

hexanes and DCM in a 1:1 ratio to yield pure compound E. ^{1}H NMR ($CDCl_3$): δ 10.03 (s, 1H, -CHO), δ 7.86 (d, 2H, -Ph), δ 7.30 (d, 2H, -Ph), δ 3.31 (s, 2H, $B_{10}H_{10}CHC$-$\underline{CH_2}$-Ph), δ 2.91 (s, 1H, carborane CH); ^{13}C NMR ($CDCl_3$): δ 191.69 (CHO), δ 143.34 (Ph), δ 135.57 (Ph), δ 130.56 (Ph), δ 129.83 (Ph), δ 42.77 (CH_2); MS (HR-ESI) $C_{10}H_{19}{}^{10}B_2{}^{11}B_8O$ (M + H) calcd 263.2439, found 263.2321.

***Attempted* 2,5-Bis-[(4-(carboranylmethyl)phenyl)-(1*H*-pyrrol-2-yl)-methyl]-1*H*-pyrrole (Compound F).** A solution of compound E (0.30 g, 1.2 mmol) and pyrrole (0.42 mL, 0.40 g, 6.0 mmol) was stirred at rt under anhydrous Ar for 5 minutes. A 5% solution of TFA (3 µL) was added slowly and stirring was continued for 15 minutes. The reaction mixture was then quenched with 0.5M NaOH (0.1 mL) and dichloromethane before being washed three times with water. The organic layer was dried over Na_2SO_4, filtered, and then evaporated. The resulting residue was purified via preparative TLC with hexane and ethyl acetate in a 6:1 ratio to give compound F in 28% yield (232 mg). Definitive ^{1}H NMR data not obtained (see Results and Discussion). MS (LR-ESI) $C_{32}H_{47}B_{20}N_3$ (M + Na) calcd 712.6, found 713.7.

***Attempted* *1*,14-Bisformyl-5,10-di[4-(carboranylmethyl)phenyl]tripyrrane (Compound G), possible dimeric pyrrole di-aldehyde.** A pink solution of the compound F (0.80 g, 1.16 mmol) in DMF (10 mL) was stirred for 5 minutes at 0°C under anhydrous Ar. Distilled $POCl_3$ (1 mL) was added drop-wise and the resulting mixture was stirred for 2.5 hours at rt. Then, 0.5 mL 0.5 M NaOH was added and stirring was continued overnight at 80-90°C. The reaction mixture was then cooled to rt, diluted with

dichloromethane, and washed three times with water. The organic layer was then dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified via Preparative TLC with hexane and ethyl acetate in a 1.5:1 ratio to give (what was thought to be) compound G in 13% yield (110 mg). For *dimer*: MS (LR-ESI) $C_{20}H_{26}B_{10}N_2O_2$ (M + Na) calcd 457.3, found 457.3.

References

1. Magda, D.; Miller, R. A. Motexafin gadolinium: a novel redox active drug for cancer therapy. Sem. Cancer. Biol. 2006, 16, 466.

2. Barth, R. F.; Coderre, J. A.; Vicente, M. G. H.; Blue, T. E. Boron neutron capture therapy of cancer: current status and future prospects. Clin. Cancer. Res. 2005; 11(11): 3987-4002.

3. Tjarks, W.; Tiwari, R.; Byun, Y.: Narayanasamy, S.; Barth, R.F.; Carboranyl thymidine analogues for neutron capture therapy; Chem. Commun., 4978 - 4991, 2007.

4. Bandyopadhyaya, A. K.; Narayanasamy, S.; Barth, R.F.; Tjarks, W.; Synthesis of novel texaphyrins containing lanthanides and boron; Tetrahedron Lett., 48, 4467-4469, 2007.

5. Miyatake, S.I.; Kawabata, S.; Kajimoto, Y. Yokoyama, K.; Doi, A.; et. al. Boron neutron capture therapy for recurrent malignant meningioma. Case report. *J. Neuro-Oncol.* 2006, 78:3, 31.

6. Webler C, Homann A, Fricke U, Lehmann J. NO donors, part 8 [1]: synthesis and vasodilating activities of substituted benzylnitrates compared to cyclohexylmethylnitrate and GTN. Eur J Med Chem. 2003 Mar 17;(38): 581-586.

7. Lee Y, Silverman RB. Traceless solid-phase synthesis of chiral 3-aryl β-amino acid containing peptides using a side-chain-tethered β-amino acid building block. Org Lett. 2000, 2:3, 303-306.

8. Endo Y, Iijima T, Yamakoshi Y, Fukasawa H, Miyaura C, Inada M, Kubo A, Itai A. Potent estrogen agonists based on carborane as a hydrophobic skeletal structure- A new medicinal application of boron clusters. Chem Biol. 2001, 8, 341-355.

9. Wei WH, Wang Z, Mizuno T, Cortez C, Fu L, Sirisawad M, Naumovski L, Magda D, Sessler JL. New polyethyleneglycol-functionalized texaphyrins: synthesis and *in vitro* biological studies. Dalton Trans. 2006, 1934-1942.

10. Mody TD, Fu L, Sessler JL. Texaphyrins: synthesis and development of a novel class of therapeutic agents. Prog Inorg Chem. 2001, 49, 551-598.

11. Hao E, Fronczek FR, Vicente MG. Carborane functionalized pyrroles and porphyrins via the Suzuki cross-coupling reaction. Chem Commun (Camb). 2006 Dec 21;(47):4900-2. Epub 2006 Oct 5.

12. Cuesta L, Tomat E, Lynch VM, Sessler JL. Binuclear organometallic ruthenium complexes of a Schiff base expanded porphyrin. Chem Commun (Camb). 2008 Aug 28;(32):3744-6. Epub 2008 Jul 1

13. Littler BJ, Miller MA, Hung CH, Wagner RW, O'Shea DF, Boyle PD, Lindsey JS. Refined Synthesis of 5-Substituted Dipyrromethanes. J Org Chem. 1999, 64(4), 1391-1396.

14. Ka, Jae-Won; Lee, Chang-Hee. Optimizing the synthesis of 5,10-disubstituted tripyrromethanes. Tetrahedron Letters (2000), 41(23), 4609-4613.

Acknowledgements

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